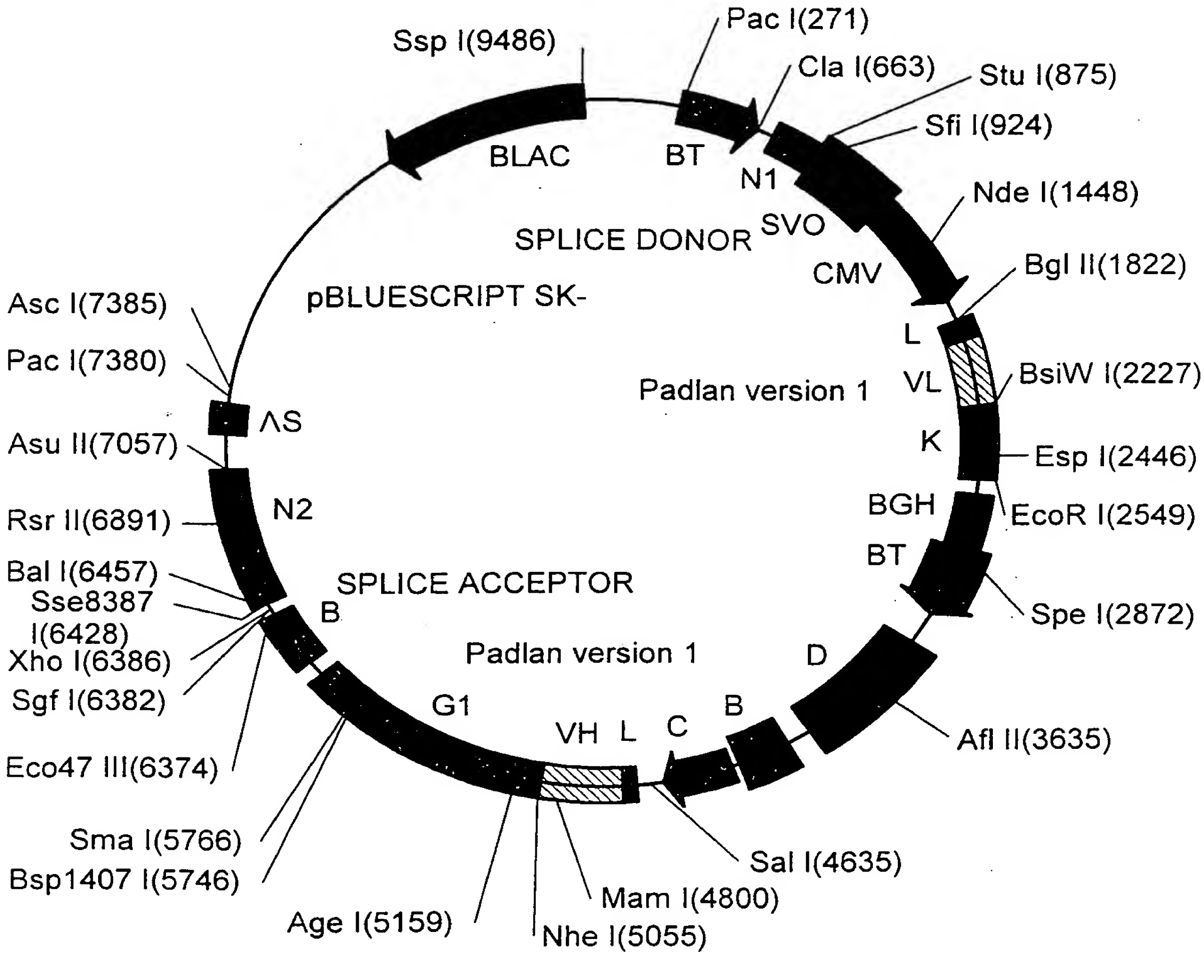
FIG. 1

Anti-GP39 HU24-31 in N5KG1 (Version #1)



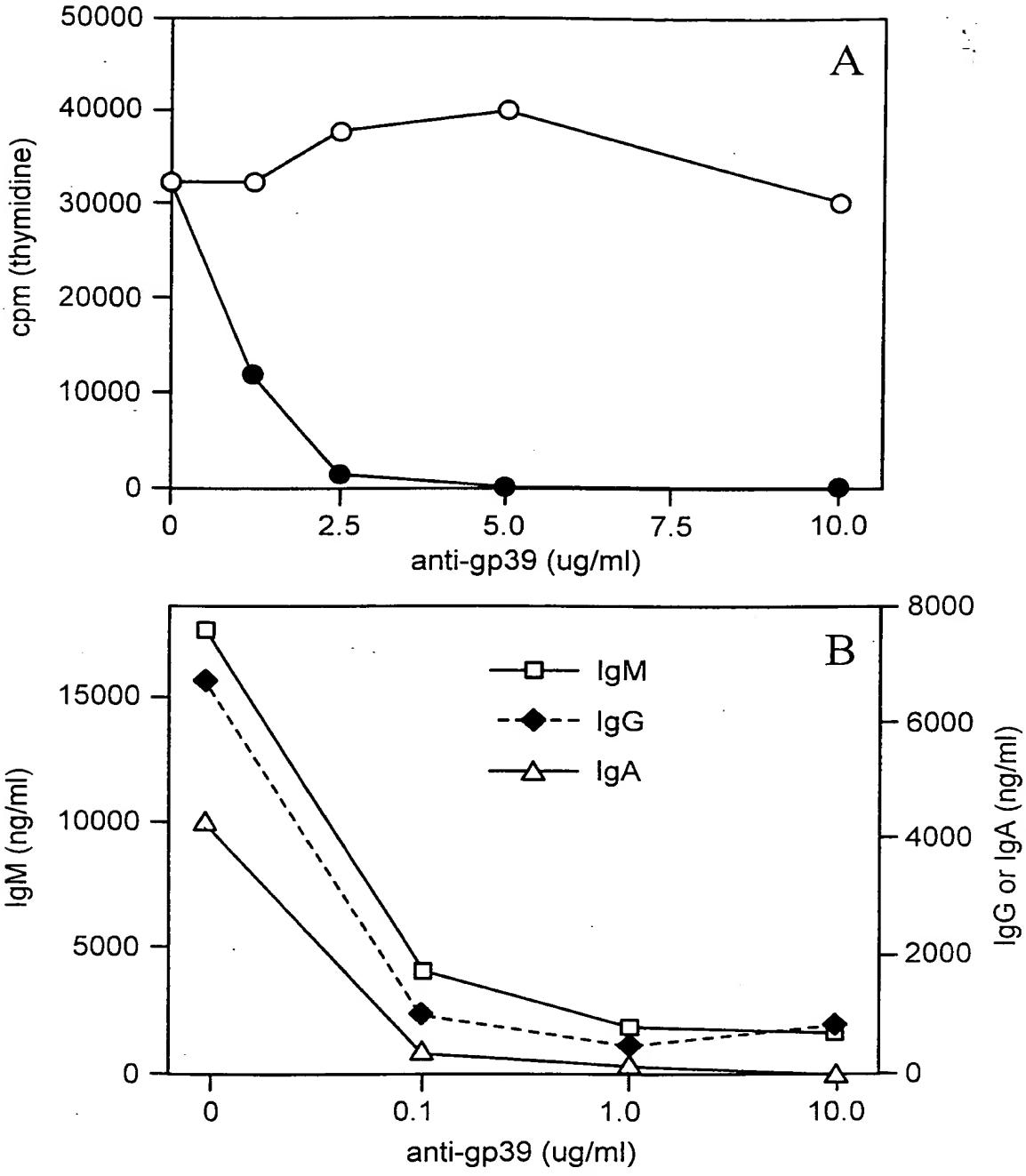
9597 BP — Map BY Mitchell Raff — 06/01/95

C = Cytomegalovirus promoter/enhancer
BT = Mouse Beta globin major promoter
B = Bovine growth hormone polyadenylation
N1 = Neomycin phosphotransferase exon 1
N2 = Neomycin phosphotransferase exon 2
K = Human immunonoglobulin kappa constant region
G1 = Human immunonoglobulin gamma 1 constant region
VL = Anti-GP39 variable light region (version #1)
VH = Anti-GP39 variable heavy region (version #1)
L = Leader
SVO = SV40 origin
SV = SV40 polyadenylation
D = Dihydrofolate Reductase
BLAC = Betalactamase gene

NONCUTTERS = AvrII, Bst1107I, DraIII, FseI, HindIII, I-PpoI, I-SceI, KpnI, MluI, MunI, PmeI, PmlI, SgrAI, SrfI, Swa I, XbaI, XcmI

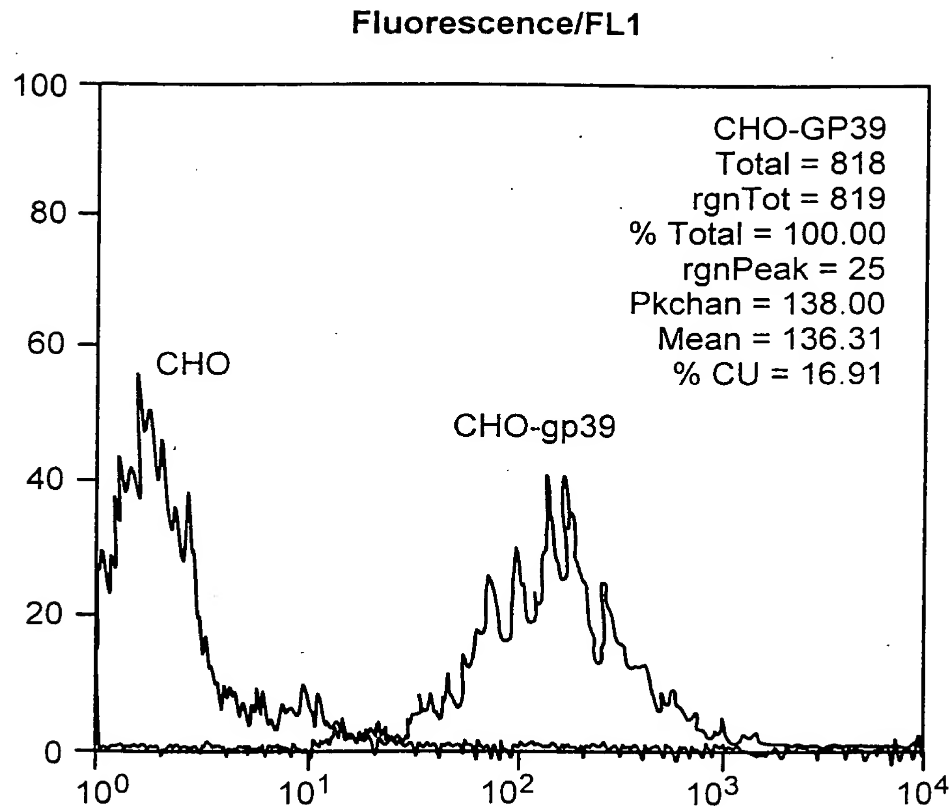
N5KG1 cut BglII + BsiWI and VL dropped in & cut SalI + NheI and VH dropped in.

FIG. 2



Anti-gp39 inhibits B cell proliferation and differentiation, but not allogeneic T cell proliferation. A. Human PBL were cultured in 96 well plates (0.1 x 10^6/well) in the presence or absence of the 20% (v/v) soluble gp39-CD8 (sgp39-CD8) fusion protein and 5 ug/ml rhuIL-4 for 3 d. Anti-gp39 mAb, 24-31 ( ), or a control murine IgG1 mAb (O), were added at a range of concentrations (1.25-10 ug/ml). Cultures with pulsed with 1 uCi^3 H-thymidine during the final 6 hr of a 72 hr culture period. B. Mitomycin treated T cells (5 x 10^4/well) activated with immobilized anti-CD3 (64.1) were cultured with 2.5 x 10^4/well IgD^+ B cells in 96-well microtiter plates for 12d in the presence or absence of various concentrations (0.1-10.0 ug/ml) of anti-gp39 mAb, 24-31. Culture supernatants were subsequently assayed for IgM□), IgG◆), and IgA△) by isotype specific ELISA.

FIG. 3



FACS analysis of non-transfected CHO cells and a gp39 transfectant. 1 X 10^6 cells were treated with the mouse anti-gp39 antibody 24-31 and then with a goat-anti-mouse IgG-FITC conjugate (Southern Biotechnology Associates). The samples were analyzed on FACScan (Becton Dickenson).

24-31 Humanized V_L #1

	BglII		9			18			27			36			45			54
5'	AGA	TCT	CTC	ACC	ATG	GGC	TTC	AAG	ATG	GAG	TCA	CAG	TTT	CTG	GCC	TTT	GTA	TTC
					M	G	F	K	M	E	S	Q	F	L	A	F	V	F

		63			72			81		FR1	90			99			108
GCG	TTT	CTC	TGG	TTG	TCT	GGT	GTT	GAT	GGA	GAC	ATT	GTG	ATG	ACC	CAG	TCT	CCA
A	F	L	W	L	S	G	V	D	G	D	I	V	M	T	Q	S	P

		117			126			135			144			153	CDR1		162
TCT	TTC	CTC	TCC	GCC	TCC	GTA	GGA	GAC	AGG	GTC	ACC	ATC	ACC	TGC	AAG	GCC	AGT
S	F	L	S	A	S	V	G	D	R	V	T	I	T	C	K	A	S

		171			180			189	FR2		198			207			216
CAG	AAT	GTG	ATT	ACT	GCT	GTA	GCC	TGG	TAT	CAA	CAG	AAA	CCA	GGA	AAG	TCT	CCT
Q	N	V	I	T	A	V	A	W	Y	Q	Q	K	P	G	K	S	P

		225			234	CDR2		243			252	FR3		261			270
AAA	TTG	CTG	ATT	TAC	TCG	GCA	TCC	AAT	CGG	TAC	ACT	GGA	GTC	CCT	GAT	CGC	TTC
K	L	L	I	Y	S	A	S	N	R	Y	T	G	V	P	D	R	F

		279			288			297			306			315			324
TCA	GGC	AGT	GGG	TCT	GGG	ACA	GAT	TTC	ACT	CTC	ACC	ATC	AGC	TCT	CTC	CAG	CCA
S	G	S	G	S	G	T	D	F	T	L	T	I	S	S	L	Q	P

		333			342			351	CDR3		360			369			378
GAA	GAC	TTC	GCA	GAT	TAT	TTC	TGC	CAG	CAA	TAT	AAC	AGC	TAT	CCG	TAC	ACG	TTC
E	D	F	A	D	Y	F	C	Q	Q	Y	N	S	Y	P	Y	T	F

	FR4	387			396			405	BsiWI		
GGA	GGG	GGG	ACC	AAG	CTG	GAA	ATC	AAA	CGT	ACG	3'
G	G	G	T	K	L	E	I	K	R	T	

FIGURE 4

24-31 Humanized V_L #2

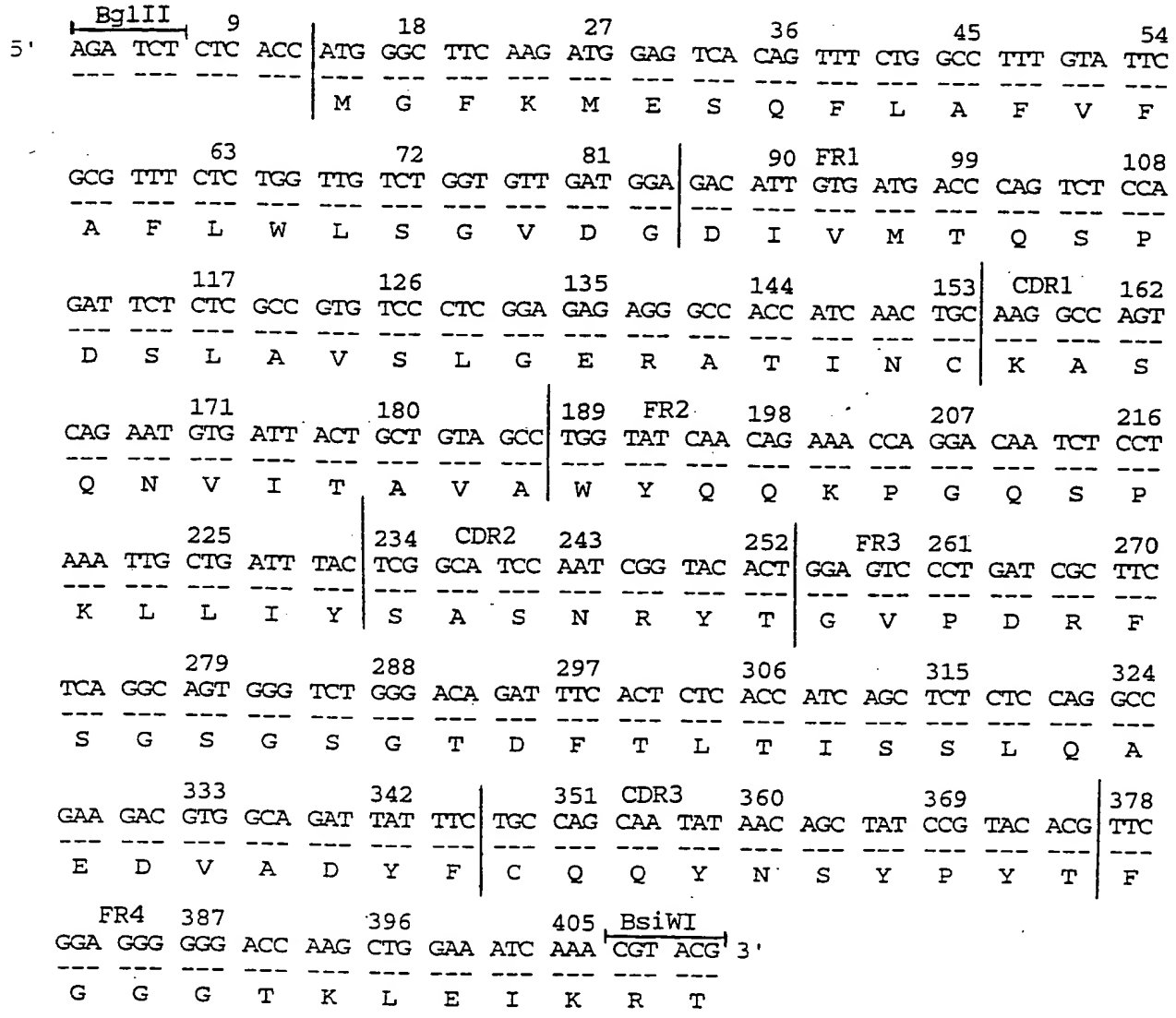


FIGURE 5

24-31 Humanized V_H #1

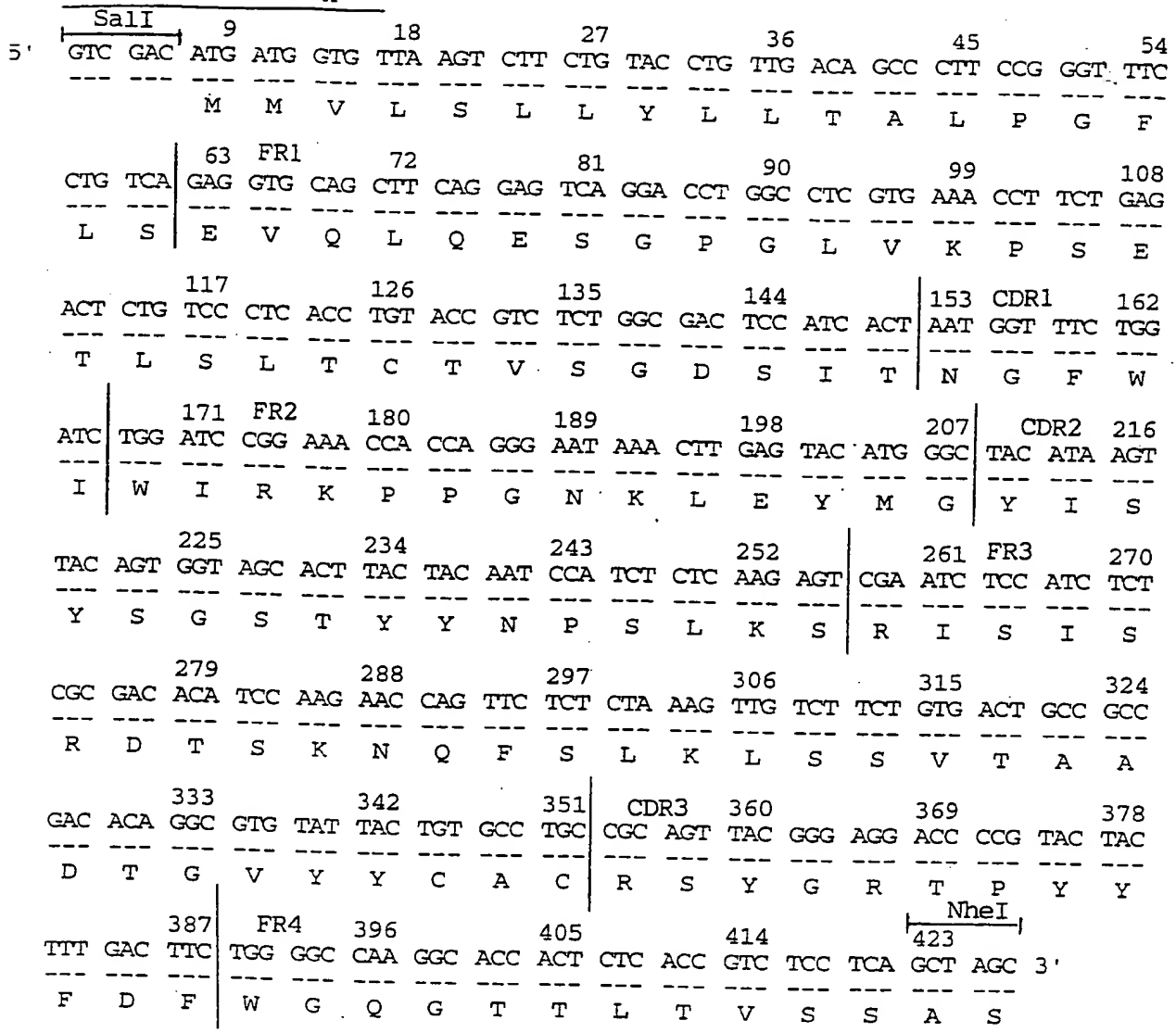


FIGURE 6

Anti-gp39 24-31 V_K Sequence

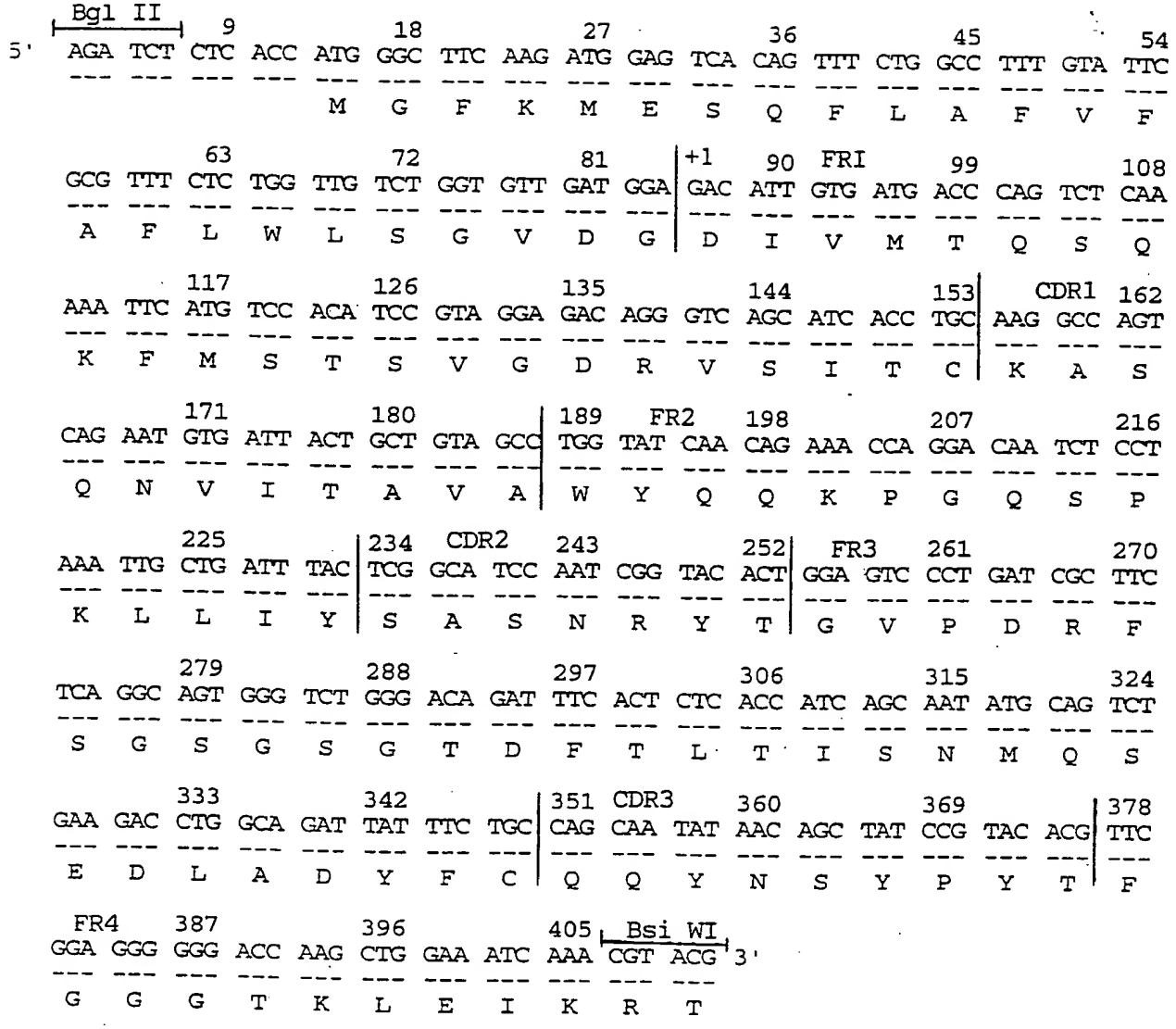


FIGURE 7

Anti gp39 24-31 V_H Sequence

	SalI		9			18			27			36			45			54
5'	GTC	GAC	ATG	ATG	GTG	TTA	AGT	CTT	CTG	TAC	CTG	TTG	ACA	GCC	CTT	CCG	GGT	TTC
			M	M	V	L	S	L	L	Y	L	L	T	A	L	P	G	F

		+1 63		FRI	72			81			90			99			108
CTG	TCA	GAG	GTG	CAG	CTT	CAG	GAG	TCA	GGA	CCT	AGC	CTC	GTG	AAA	CCT	TCT	CAG
L	S	E	V	Q	L	Q	E	S	G	P	S	L	V	K	P	S	Q

		117			126			135			144			153	CDR1		162
ACT	CTG	TCC	CTC	ACC	TGT	TCT	GTC	ACT	GGC	GAC	TCC	ATC	ACT	AAT	GGT	TTC	TGG
T	L	S	L	T	C	S	V	T	G	D	S	I	T	N	G	F	W

		171	FR2		180			189			198			207		CDR2	216
ATC	TGG	ATC	CGG	AAA	TTC	CCA	GGG	AAT	AAA	CTT	GAG	TAC	ATG	GGC	TAC	ATA	AGT
I	W	I	R	K	F	P	G	N	K	L	E	Y	M	G	Y	I	S

		225			234			243			252			261			FR3 270
TAC	AGT	GGT	AGC	ACT	TAC	TAC	AAT	CCA	TCT	CTC	AAG	AGT	CGA	ATC	TCC	ATC	ACT
Y	S	G	S	T	Y	Y	N	P	S	L	K	S	R	I	S	I	T

		279			288			297			306			315			324
CGC	GAC	ACA	TCC	CAG	AAC	CAG	TTC	TAC	CTA	CAA	TTG	AAT	TCT	GTG	ACT	ACT	GAG
R	D	T	S	Q	N	Q	F	Y	L	Q	L	N	S	V	T	T	E

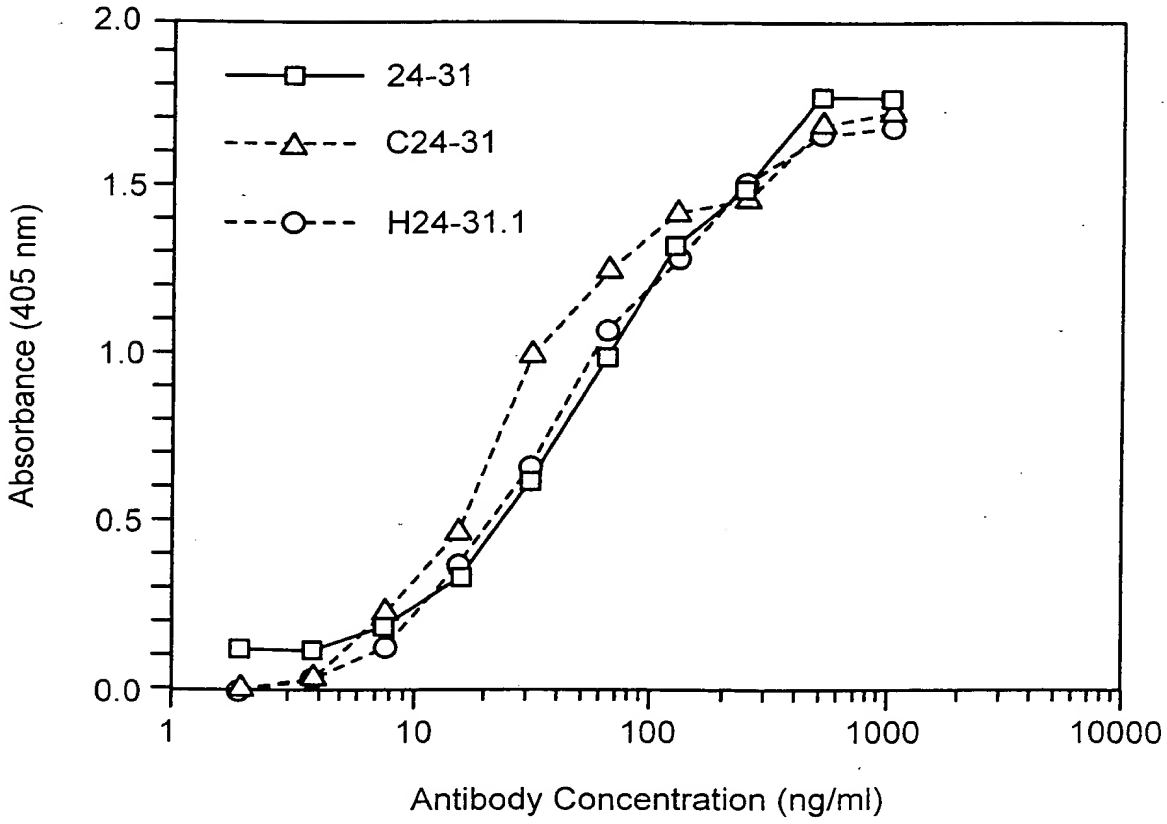
		333			342			351	CDR3		360			369			378
GAC	ACA	GGC	ACA	TAT	TAC	TGT	GCC	TGC	CGC	AGT	TAC	GGG	AGG	ACC	CCG	TAC	TAC
D	T	G	T	Y	Y	C	A	C	R	S	Y	G	R	T	P	Y	Y

		387	FR4		396			405			414			423	NheI	
TTT	GAC	TTC	TGG	GGC	CAA	GGC	ACC	ACT	CTC	ACC	GTC	TCC	TCA	GCT	AGC	3'
F	D	F	W	G	Q	G	T	T	L	T	V	S	S	A	S	

FIGURE 8

FIG. 9

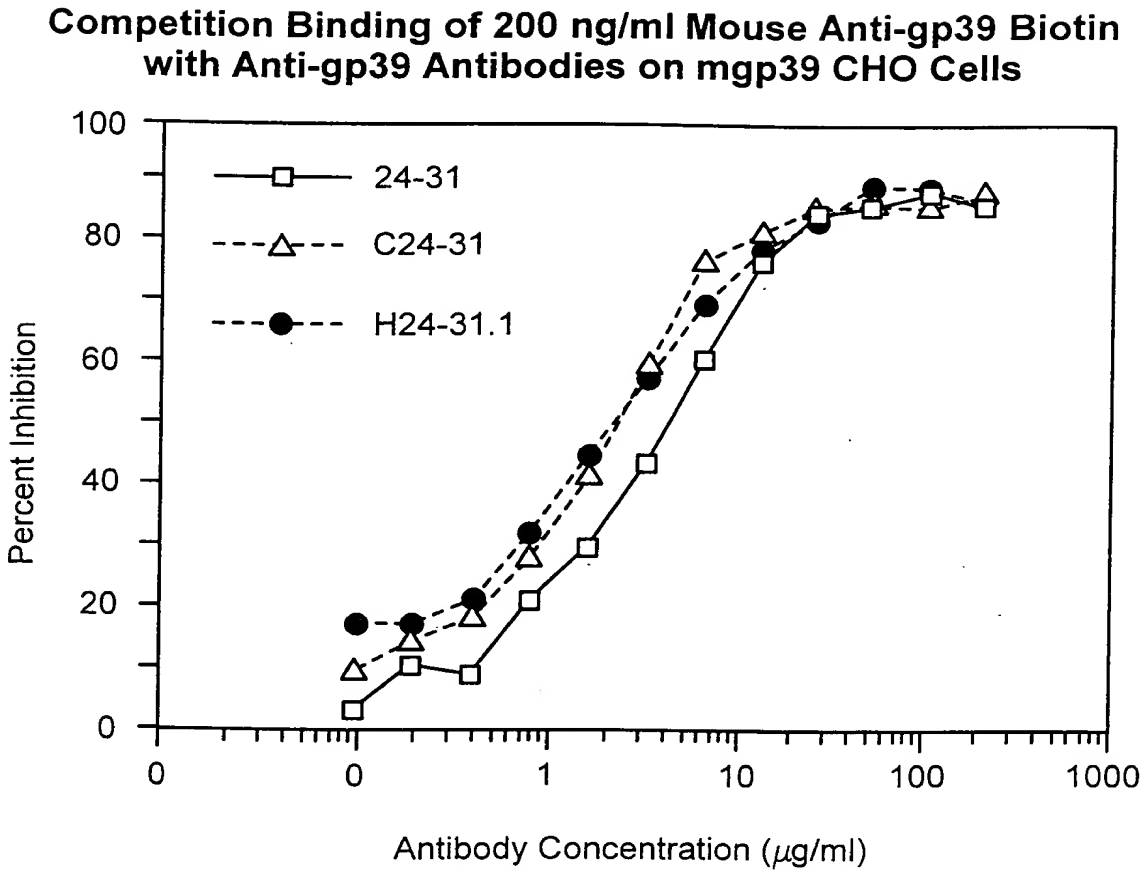
Direct Binding of Anti-gp39 Antibodies to mgp39 CHO Cells



Fifty ul of 1 ug/ml solution of each anti-gp39 antibody (murine, chimeric and humanized version 1 of 24-31) was added to wells containing poly-l-lysine fixed mgp39 CHO cells. After a 2 hour incubation, the bound antibodies were detected with either goat anti-human IgG HRP or goat anti-human IgG HRP or goat anti-mouse IgG HRP. The binding capacity of each antibody was compared on a plot of absorbance vs antibody concentration.

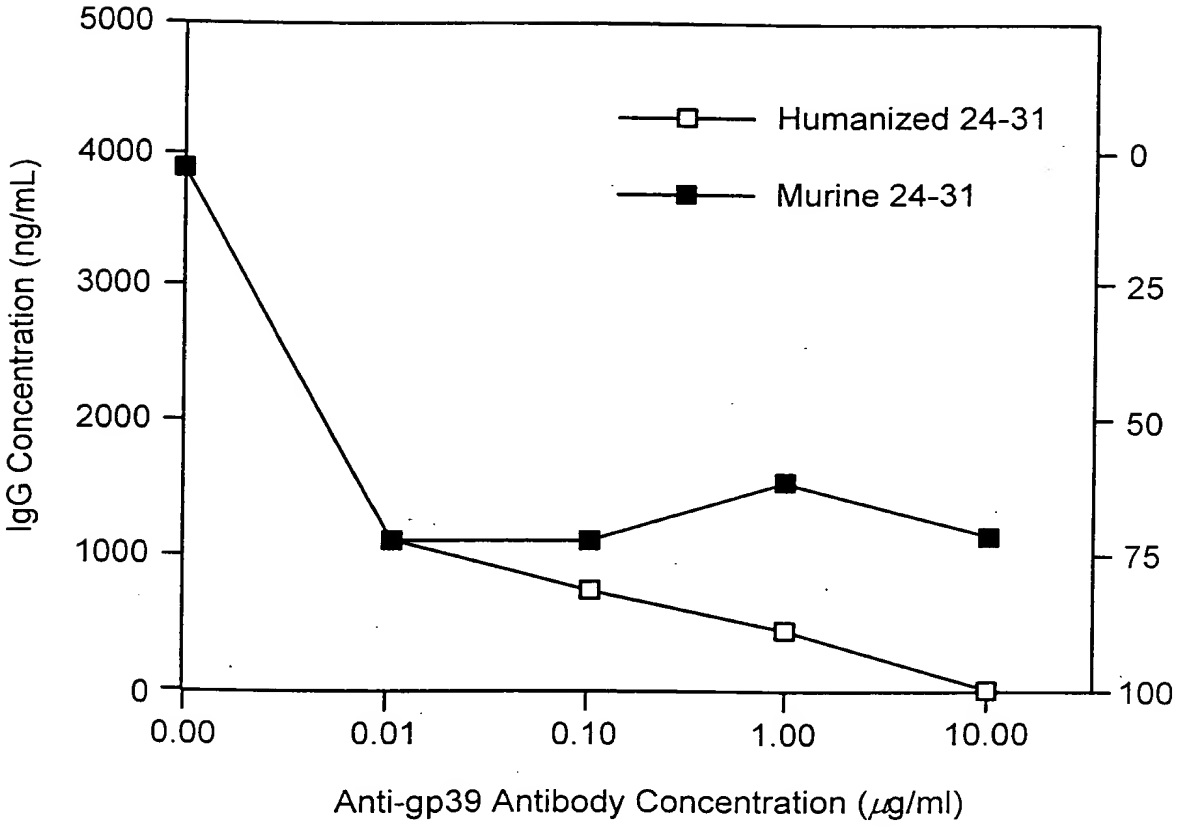
The figure shows that the half maximal binding in ELISA is achieved at similar concentrations for all three versions, at approximately 40 ng/ml.

FIG. 10



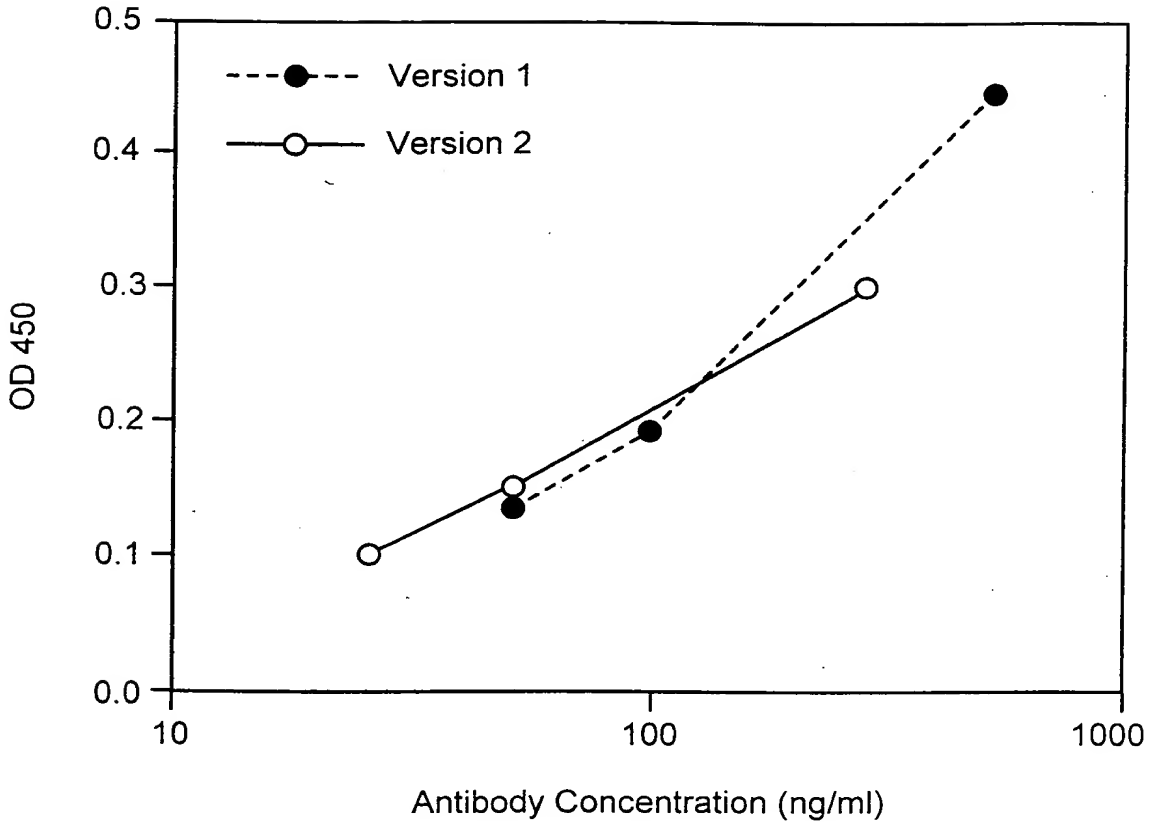
The figure shows that competition for binding to gp39 between biotin labeled 24-31 and the mouse, chimeric and humanized versions 1 are comparable, possibly with the humanized version slightly better than the original antibody, with half-maximal competition at 2 and 4 μg/ml, respectively.

FIG. 11



Purified, mitomycin C treated T cells were added into cell culture plates coated with anti-CD3 antibody. Autologous purified B cells were mixed with antibody at described concentrations and added to these plates in regular growth media. After 10 days the supernatant was tested for content of human IgM.

FIG. 12



CHO cell supernatant containing humanized 24-31 version 1 and version 2 in unknown amounts, was incubated on mgp39-CHO cells for 2 hours. After a wash, the amount of bound antibody was determined. The same supernatants were tested in parallel on an ELISA plate coated with Goat α Human γ, to determine the concentration of human IgG present relative to a control of known concentration. The binding data were normalized relative to the total antibody concentration.

FIG. 13

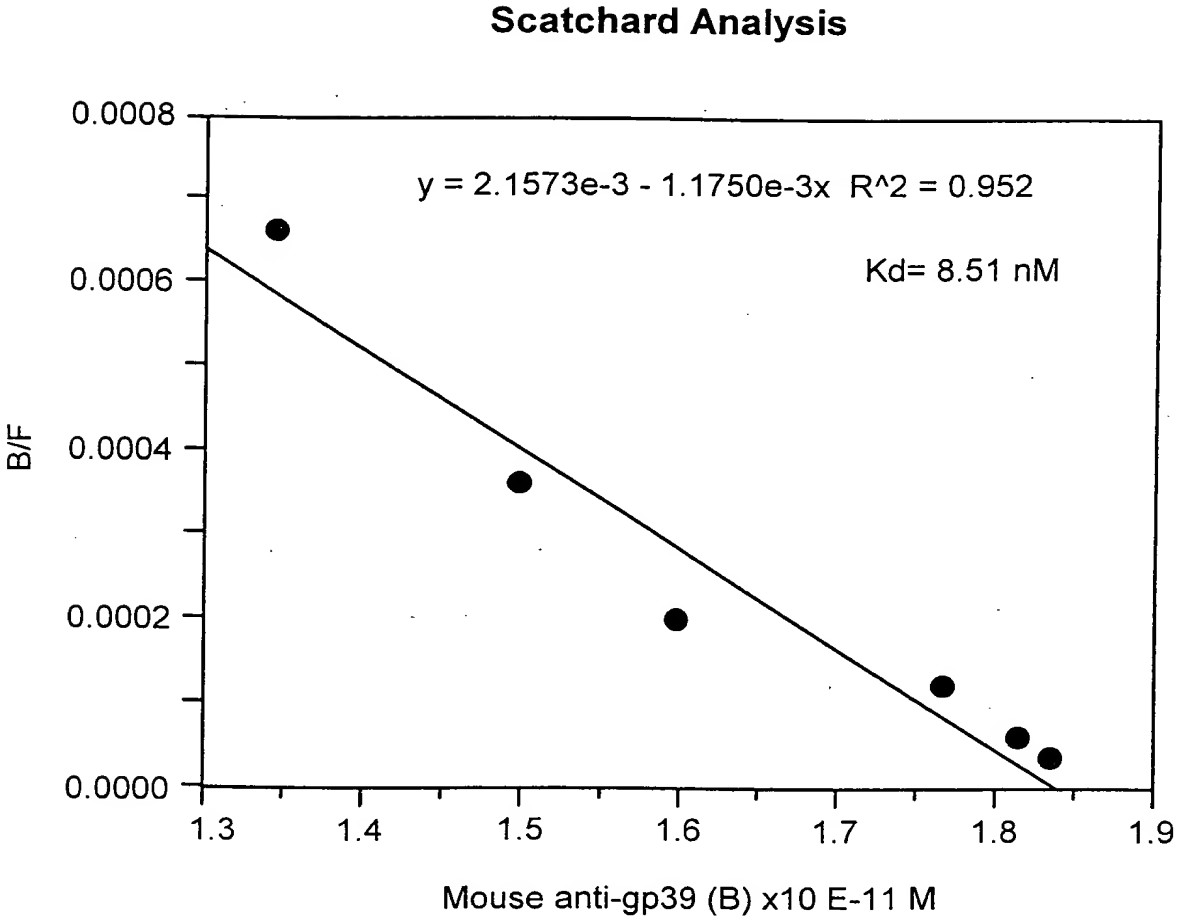

Scatchard Analysis
y = 2.1573e-3 - 1.1750e-3x R^2 = 0.952
Kd= 8.51 nM
B/F
0.0008
0.0006
0.0004
0.0002
0.0000
1.3
1.4
1.5
1.6
1.7
1.8
1.9
Mouse anti-gp39 (B) x10 E-11 M

FIG. 14

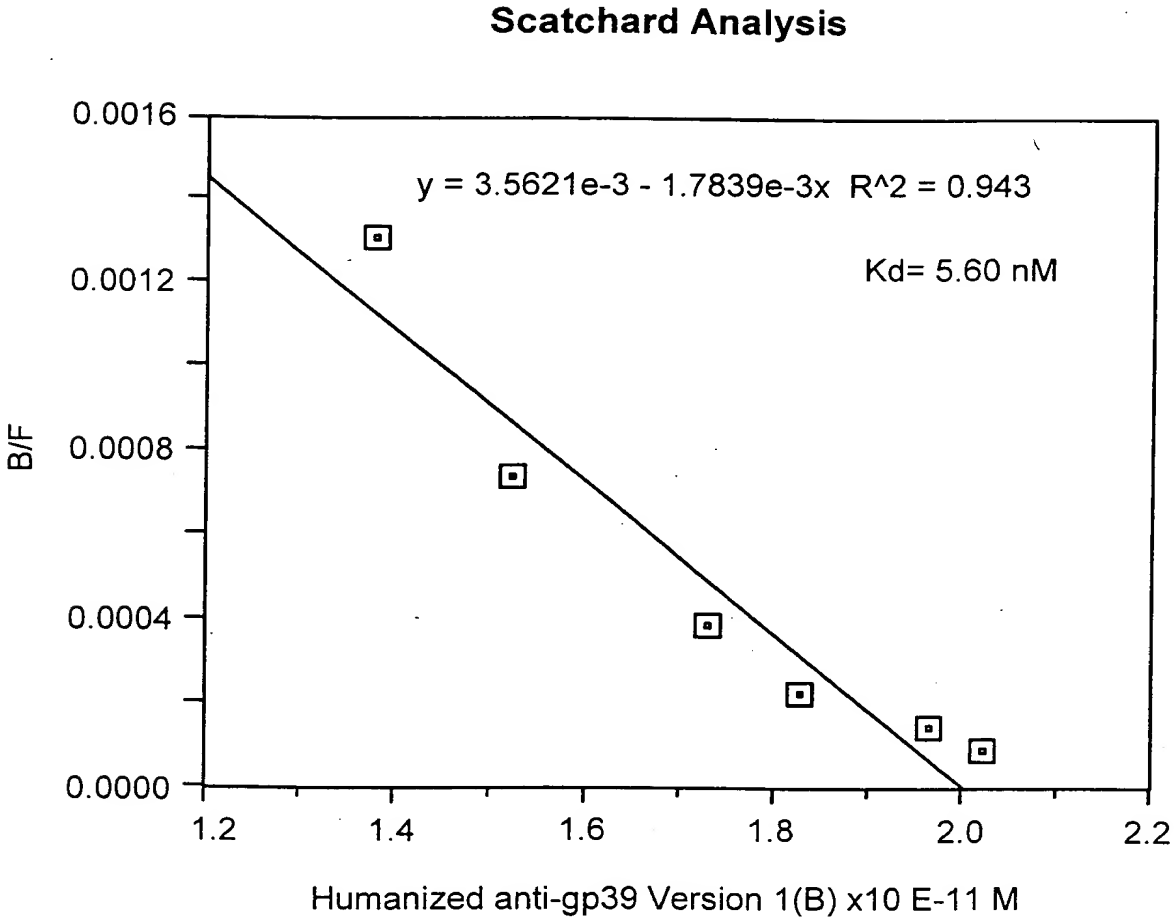
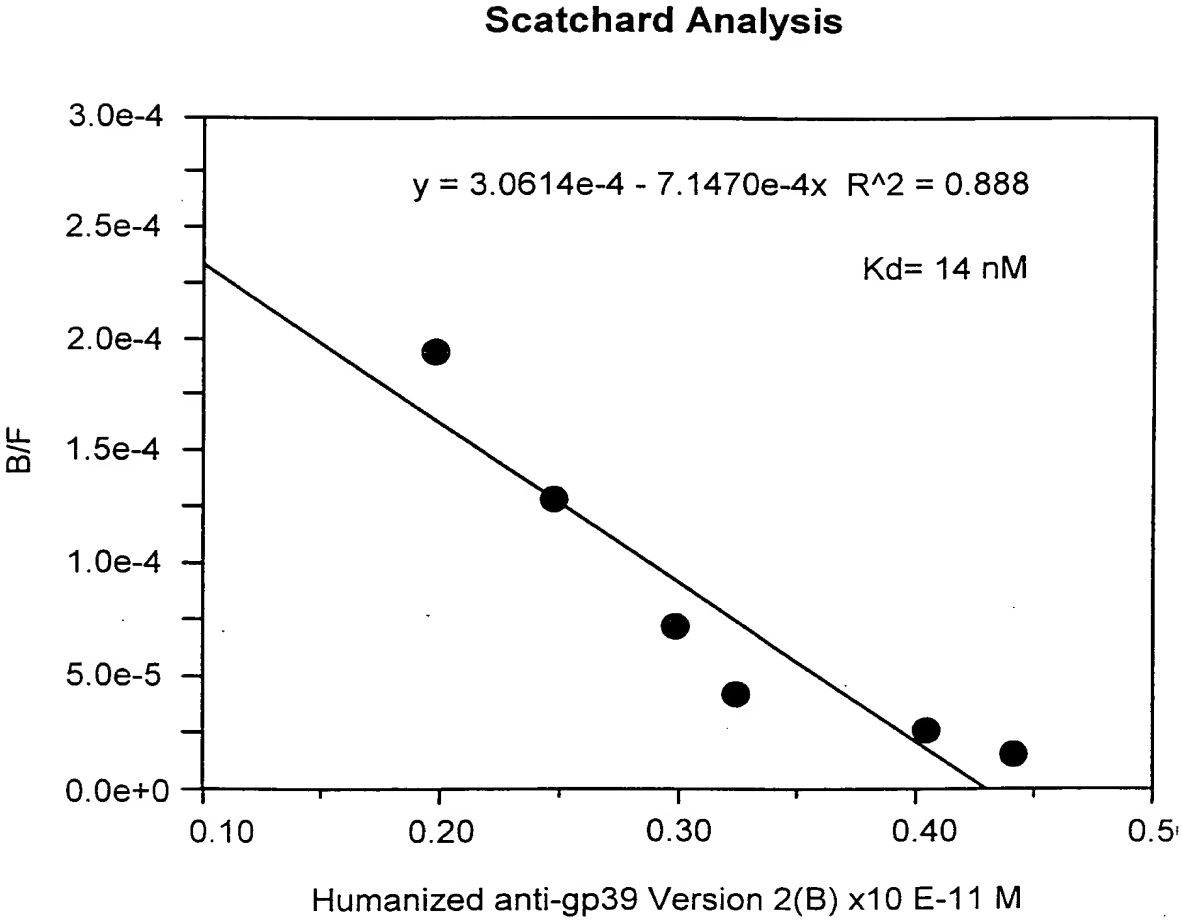

Scatchard Analysis
y = 3.5621e-3 - 1.7839e-3x R^2 = 0.943
Kd= 5.60 nM
B/F
0.0016
0.0012
0.0008
0.0004
0.0000
1.2
1.4
1.6
1.8
2.0
2.2
Humanized anti-gp39 Version 1(B) x10 E-11 M

FIG. 15


Scatchard Analysis
y = 3.0614e-4 - 7.1470e-4x R^2 = 0.888
Kd= 14 nM
B/F
3.0e-4
2.5e-4
2.0e-4
1.5e-4
1.0e-4
5.0e-5
0.0e+0
0.10
0.20
0.30
0.40
0.5
Humanized anti-gp39 Version 2(B) x10 E-11 M

Soluble Anti-CD40L Antibody (TRAP1) Stimulates IL-2 Cytokine Release from CD4+ T Cells in Presence of Immoblilized Anti-CD3

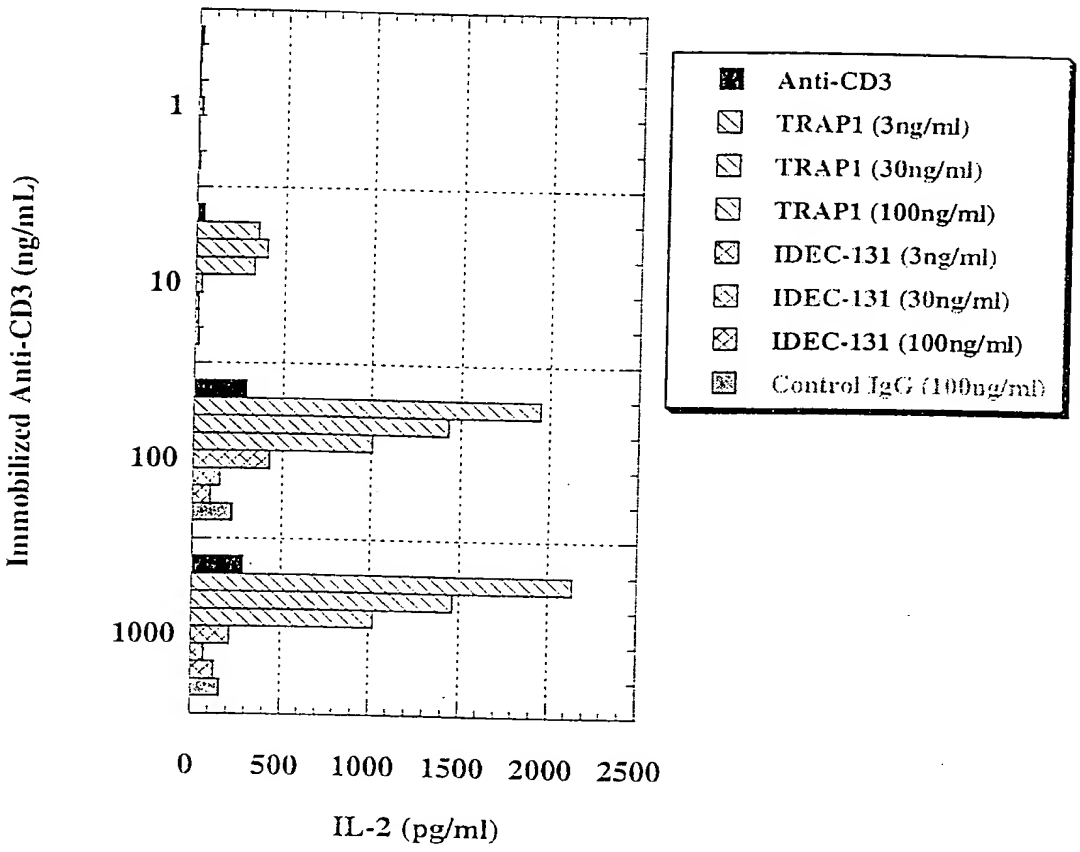


Figure 16

Soluble Anti-CD40L Antibody (TRAP1) Stimulates IL-4 Cytokine Release from CD4+ T cells in Presence of Immobilized Antii-CD3

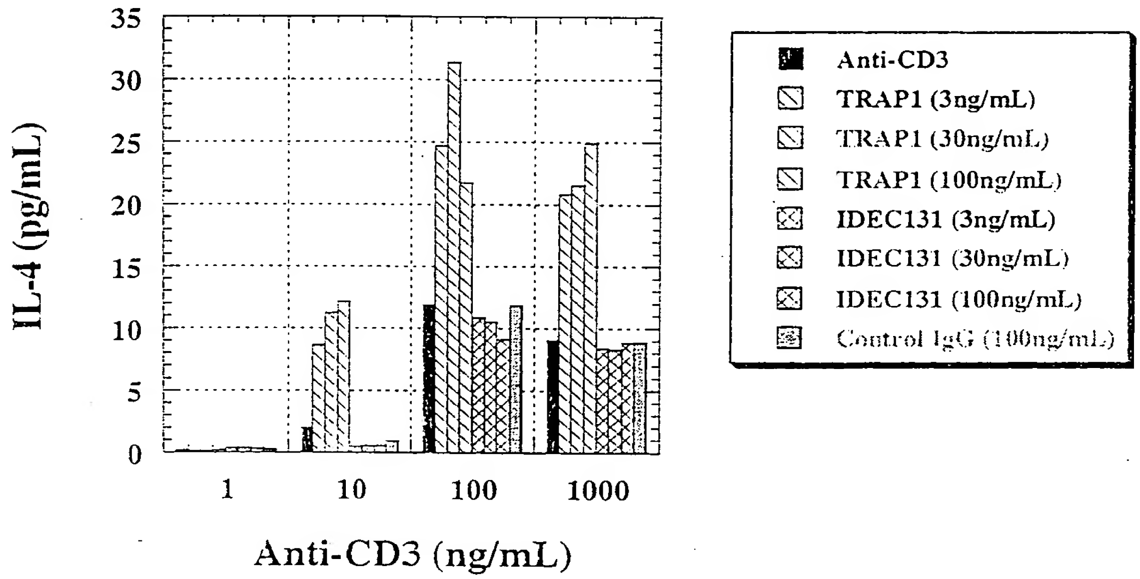


Figure 17

Stimulation of IL-2 by Agonist TRAP1 Anti-CD40L is Dependent on Co-stimulation with Anti-CD3 and Signaling Through CD40L

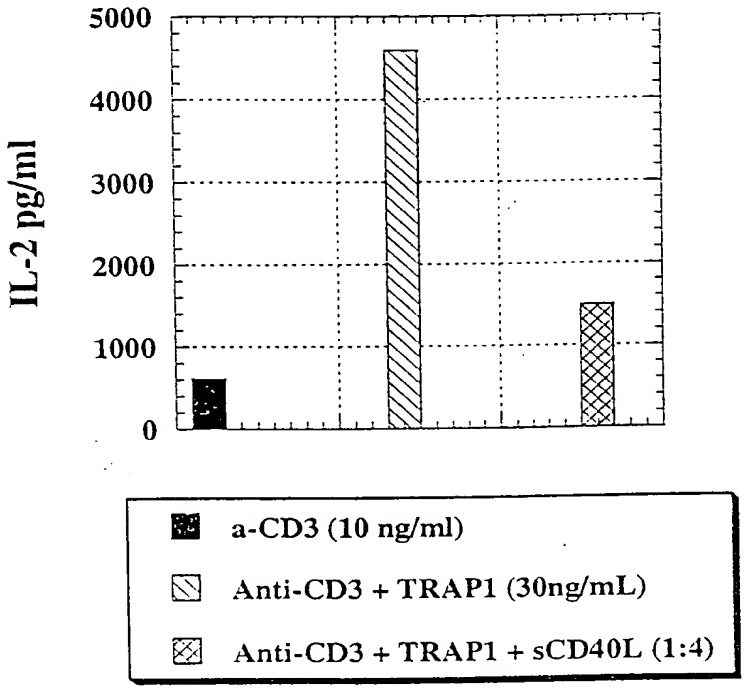


Figure 18

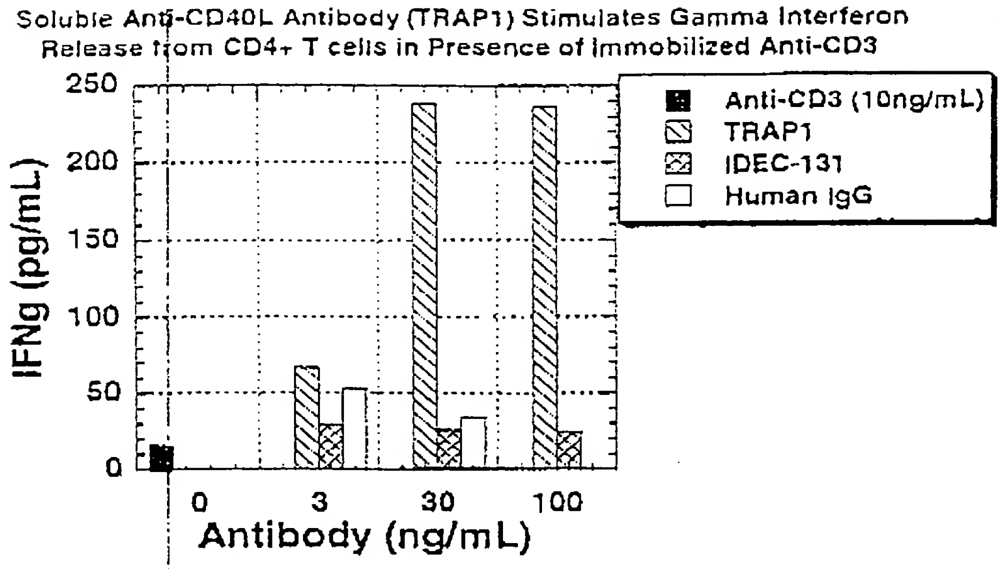


Figure 19

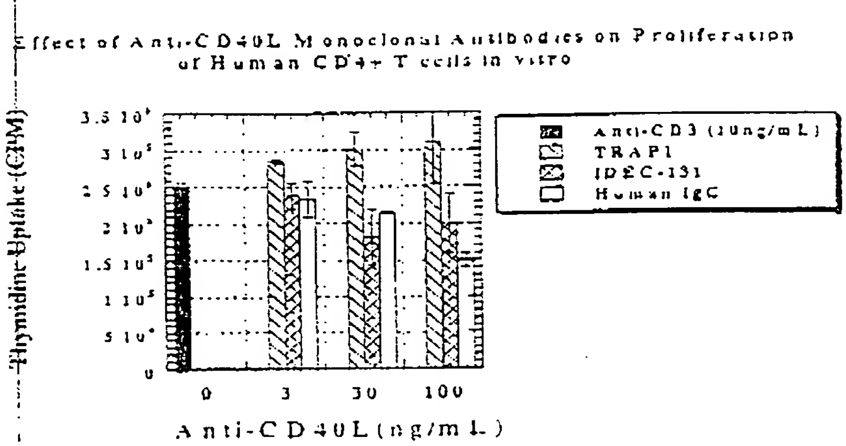


Figure 20